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A Two-Year Study on Milk Quality from Three Pasture-Based Dairy Systems of Contrasting Production Intensities in Wales

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SUMMARY

There is an increasing interest in pasture-based dairy systems in Europe mainly because of increasing production costs for intensive dairying. Milk is a matrix of compounds that influence nutritional and manufacturing properties, many dependent on husbandry linked to pasture-based systems (increase in pasture intake, forage:concentrate ratio, clover inclusion in swards/silages and use of alternative dairy breeds). This study investigated the impact of three grazing-based dairy systems with contrasting feeding intensity or reliance on pasture intakes, (conventional high-intensity, low pasture intake [CH]; organic medium-intensity, medium pasture intake [OM], conventional low-intensity, high pasture intake [CL]) on milk fatty acid (FA) profiles, protein composition and α-tocopherol and antioxidants contents. The proportion of animals of alternative breeds (e.g. Jersey) and crossbred cows in the herd increased with decreasing production intensity (CH < OM < CL). Milk constituents known to be beneficial for human health, such as vaccenic acid, rumenic acid, monounsaturated FA, polyunsaturated FA, antioxidants and caseins, were elevated with decreasing production intensity (CH < OM < CL), while less desirable saturated FA were lower, although not all differences between OM and CL were significant. Omega-3 FA were maximised under OM practices, primarily as a result of higher clover intake. Increases in pasture intake may explain the higher desirable FA contents while increased use of crossbreed cows is likely to be responsible for higher total protein and casein content of milk; a combination of these two factors may explain increased antioxidant content. The higher concentrations of vaccenic acid, rumenic acid, omega-3 FA, lutein, zeaxanthin, protein and casein in OM and CL milk were found over most sampling months and in both years, reinforcing the higher nutritional quality and manufacturing properties associated with milk from these systems.. A switch to pasture-based dairy products would increase the intake of milk's beneficial compounds and reduce consumption of less desirable saturated FA.

Keywords: pasture-based, milk, fatty acid, protein, antioxidants

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INTRODUCTION

Milk and dairy products have always been a key component in human diet largely because they deliver a matrix of nutritional compounds including fat, protein, vitamins, antioxidant and minerals (Haug *et al.* 2007; Kratz *et al.* 2013). Milk fat is mainly composed of fatty acids (FA; about 0.933 of total fat) some of which are beneficial for human health. These include monounsaturated FA (MUFA) vaccenic acid (VA; t11 C18:1) and oleic acid (OA; c9 C18:1), and polyunsaturated FA (PUFA) omega-3 (n-3), omega-6 (n-6) and rumenic acid (RA; c9t11 C18:2 conjugated) (Givens & Shingfield 2004; Kliem & Givens 2011). However, individual saturated FA (SFA) in milk, such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) have been associated with increased risk of cardiovascular diseases (CVD) (Givens & Shingfield 2004; Kliem & Givens 2011). Milk proteins consist of caseins (about 800 g/Kg of protein) and whey (about 200 g/Kg of protein) proteins and serve a dual role of (i) being essential nutritional components by providing amino acids and bioactive peptides (Severin & Wenshui 2005; Korhonen & Pihlanto 2006; Madureira *et al.* 2007) and (ii) controlling the cheese making properties of milk (Christian *et al.* 1999; Wedholm *et al.* 2006). Tocopherols and antioxidants play a number of roles in milk; (i) improve health status of dairy cows (Givens & Shingfield 2004), ii) protect milk proteins and lipids from oxidation (Lindmark-Mansson & Akesson 2000; Havemose *et al.* 2004) and (iii) offer cellular oxidation protection for consumers (Haug *et al.* 2007). Although milk is far from being considered a crucial source of antioxidants in our diet since green leafy vegetables, herbs, leek, peas, broccoli, carrots and eggs provide considerably more (Maiani *et al.* 2009; Abdel-Aal *et al.* 2013). Despite emerging evidence of a positive association between dairy consumption and protection against CVD, diabetes (Kratz *et al.* 2013) or certain cancers (colorectal, and possibly bladder) (Kliem & Givens 2011), dietary recommendations aim to reduce dairy fat consumption and replace dairy fat with less saturated plant derived oils (Kliem & Givens 2011; Kratz *et al.* 2013).

Recently, there is a resurgence of interest among producers in pasture-based dairying in UK and Ireland, driven by economic benefits stemming from reduced reliance on more expensive concentrate feeds.. Grazing is the cheapest source of nutrients for ruminants; estimated production costs decrease by 0.01€/L milk for every 2.5% increase in fresh grass in dairy diets (O'Donovan *et al.* 2011). At the same time, there is growing evidence that reduced intensification of dairy production, especially feeding (a greater emphasis on grazing , forage:concentrate ratio or both) may increase the content of compounds beneficial for human health (Butler *et al.* 2008; Stergiadis *et al.* 2012). Feeding cows fresh herbage instead of conserved forage and concentrate feeds has been shown to improve the nutritional composition of milk by increasing beneficial n-3, MUFA, PUFA and RA, vitamins and antioxidants and by decreasing SFA (Butler *et al.* 2008; Slots *et al.* 2009; Stergiadis *et al.* 2012). Other management practices adopted in pasture-based systems, such as (i) including legumes in grazing and conservation swards, especially in organic farming where nitrogen fertilisers are not permitted (Butler *et al.* 2008; Dewhurst *et al.* 2009), (ii) using smaller, alternative breeds, like Jersey, for crossbreeding with Holstein/Friesian cows to improve stocking rate, welfare, fertility and calving ease (Weigel & Barlass 2003; Sorensen *et al.* 2008) may influence milk composition. For example, clover has been associated with an increase in n-3 in milk (Dewhurst *et al.* 2009;

Lee *et al.* 2009) while Jersey milk is higher in total SFA, protein, casein and casein:whey protein ratio compared with that from Holstein/Friesian cows (Carroll *et al.* 2006; Stergiadis *et al.* 2013).

Given its complexity, composition and multiple processing applications, milk should be studied as a matrix of compounds and investigations ought to consider a range of components with respect to its nutritional and manufacturing value. This study (i) assesses the impact of grazing/management systems on a broad range of nutritionally relevant milk components (FA profile, protein composition, α-tocopherol and antioxidant content), (ii) investigates the persistency of outcomes over two sampling periods and (iii) associates other management parameters (e.g. crossbreeding and sward botanical composition) with nutritionally relevant components in milk by multivariate analyses.

MATERIALS AND METHODS

Experimental Design

The survey was conducted in the south-west Wales on 15 farms representing three pasture-based production systems widely used in this region. All farms operated paddock grazing with contrasting reliance on grazed herbage, conserved forage and supplementary feeds in dairy diets (conventional high-intensity, low pasture intake [CH] < organic medium-intensity, medium pasture intake [OM] < conventional low-intensity, high pasture intake [CL]) with five farms for each system. Milk yield and composition along with diet details and management practices were recorded/collected on eight occasions over two 10-month periods (i) August 2004, October 2004, March 2005, May 2005 (year 1) and (ii) March, May, June, August, October 2007 (year 2). Milk samples representing 24 hour production were collected from the bulk tank (after stirring) and kept frozen at -20 °C until analysis. On each sampling date questionnaires were completed by the farmers providing herd details (numbers of lactating cows and heifers in the herd and those calving since the last collection), milk yield, feed composition (type and amounts of conserved forage, cereals and concentrate feeds) and other supplements (minerals/vitamin and oil), housing and grazing management, signs of and treatment for mastitis and other ailments. Data on the gross composition of milk (fat, protein, lactose, urea content and somatic cell count (SCC) obtained from routine milk recording) along with herbage from the grazing swards were collected. This information, together with data on the genetics/breed composition of the herd was used to define the husbandry/management practices. Estimated herd dry matter intake (DMI) and pasture intake (by difference) were calculated as described by Butler et al. (2008) using milk yield, breed and feed composition and quantity data collected via the farmer questionnaire. Details of feeding regimes, milk yields and gross composition and other production system parameters are presented in Table 1. The main characteristics of the three pasture-based production systems in this study were:

1. ***Conventional High-Intensity (CH).*** Five farms representing common conventional management with a grazing allowance in Wales were included. Cows had access to grazing during summer and housed indoors during winter. Grazing and silage swards were all predominantly pure ryegrass (clover may appear in < 20 g/kg of herbage DM) and paddock rotation varied between 2-5 days depending on herbage supply and demand. All cows in CH farms were Holstein/Friesian, calving all-year-round, resulting in cows at all stages of lactation being milked throughout the year. These farms are characterised by high yield of herbage production, averaging 1.8 tDM/ha when cows were introduced to paddocks. Although described as “high-intensity”, this is a relative term within this study because highly intensive farms providing over 500 g/kg DMI from concentrates, milking 3 times per day and with limited access to grazing, as previously described for UK (Stergiadis *et al*. 2012), were not included.

2. ***Organic Medium-Intensity (OM)***. Five farms representing organic management (certified either by Soil Association or Organic Farmers and Growers) were included. Swards and silages used were mainly mixed ryegrass/clover (average composition, 870 g/kg ryegrass, 105 g/kg clover and 25 g/kg other plants on a fresh weight basis, although this did fluctuate considerably throughout the study). No mineral nitrogen or soluble P fertilizers were applied, although finely ground rock phosphate was used when necessary, based on soil analyses. As expected, in the absence of N fertiliser, herbage yield when cows were introduced into paddocks at 1.4tDM/ha, was slightly lower than in CH. Cows grazed when the conditions allowed (March-October) generally spending 1-2 days on each paddock. Herds block calved all cows between late February and April. Herd composition throughout the survey averaged 69% Holstein/Friesian, 24% Jersey or Jersey crossbreds (14% and 10% respectively) and 7% of cows of “Red” genotypes (Scandinavian Red, 3%; Ayrshire, 2%; Shorthorn, 2%).

3. ***Conventional Low-Intensity (CL)***. Five farms representing conventional pasture-based systems were included. All farms used the New-Zealand type production system (McCall & Clark 1998). Swards and silages were predominantly pure ryegrass, with clover contributing < 10 g/kg of herbage DM, and cows grazed throughout the lactation with concentrates or other supplements given at less than 100 g/kg DMI. Paddock rotation was mostly on a daily basis with cows moving to fresh grazing every day and the recorded herbage yield on introduction of 1.3 tDM/ha is slightly lower than in CH and similar to the OM system. All farms block calved between late February and April. Mineral N fertilizers up to 120 kg N/ha per year were used and water-soluble P fertilizers at detectable amounts by soil analysis were applied. Herd composition throughout the survey averaged 61% Holstein/Friesian and 39% Jersey or Jersey crossbreds (16% and 23% respectively) while a small number of cows were Ayrshire or Ayrshire crossbreds (< 0.5%).

Forage Production and Botanical Composition

Herbage details only relate to year 2 of this study. Monthly samples were collected on each farm, from 3 locations within the next paddock in the rotation, representing herbage cows would have access to the following day. Vegetation within a 0.75 x 0.75 m quadrate, thrown at random, was cut to 10 mm residual height, 3 samples were bulked and weighed then transferred to the field station. They were thoroughly mixed before a representative sub-sample was separated into: grasses, legumes and other plants and each fraction was weighed fresh and again after 12 hours forced-air oven drying at 80 ºC.

Milk Analysis

Milk FA analysis was performed following routine procedures of the laboratories of Aarhus University, Denmark, (year 1) and Newcastle University, UK, (year 2) as described by Slots et al. (2009) and Butler et al. (2011) respectively. Milk was analysed for protein profile as described by Stergiadis et al. (2012). Determination of milk α-tocopherol/antioxidant content was performed as shown by Slots et al. (2009).

Statistical Analysis

Variables expressed as proportions or percentages (individual FA, SFA, MUFA, PUFA) were arcsine transformed prior to analyses of variance (ANOVA), in common with previous work (Butler *et al.* 2011; Stergiadis *et al.* 2012; Stergiadis *et al.* 2013), but all results presented are un-transformed values. The proportions of conserved forage, grass silage, maize silage and by-products in the diet and mineral supplementation (g/cow/day) were cube root transformed, prior to ANOVA whilst other measurements were analysed untransformed. Values for FA are expressed as g/kg of total identified FA, individual protein content as g/kg milk and antioxidants and α-tocopherol content as mg/kg milk fat. Data collected in June 2007 (year 2) for milk yield, basic composition, FA profiles and milk antioxidants content were excluded from this statistical analysis because corresponding samples from 2004/2005 (year 1) were unavailable; they were however used in the ANOVA of protein composition that was assessed only during year 2 of the study. ANOVA were derived from linear mixed-effects models (Pinheiro & Bates 2000) using management (conventional high-intensity [CH], organic medium-intensity [OM], conventional low-intensity [CL]), sampling month (March, May, August, October) and year (first and second) as fixed factors and farm as the random factor. A similar model using management (CH, OM and CL), sampling date (March, May, June, August, October) as fixed factors and farm as the random factor was used to assess protein composition of milk only on year 2 Differences between means discussed as significant ($P < 0.05$) in this study refer to those identified by Tukey's honest significant difference test, used for pairwise comparisons of means. Analyses were performed in the R statistical environment (R Development Core team 2009) and residual normality was assessed using the qqnorm function (Crawley 2007), with no data showing deviation from normality. Supplemented lipid (mainly calcium salts of palm oil) was provided in one CH farm during year 2 of the study on 4 occasions (38 g per cow per day on average) although data were insufficient to carry out factorial analysis for this variable.

Multivariate redundancy analyses (RDA) allowed the relationships between multiple variables influencing responses to be assessed in datasets containing both (i) measured variables, in this case milk composition parameters and (ii) variables thought to influence these responses. This contrasts with factorial analyses where each response variable is treated separately. In this study, RDA were used to investigate the effects of variables related to sward yield, botanical composition, individual feed components and cows' breed (the 'drivers') on milk FA and protein profiles, antioxidant content, SCC and mastitis cases (the 'response variables'). RDA produce biplots where arrows indicate the relative effects of 'drivers' in relation to the 'response variables'. Sward related components were dry matter yield per hectare (DMHA), the proportions of grasses (GRP), red/white clover (CLP) and other plants (OTP) in the herbage. Feed components were dietary proportions for predicted pasture intake (GRA), grass silage (GS), maize silage (MS), other silage (OS), hay/straw (HS), cereals (CER), by products (BP; products from food and drink manufacture, typically sugar beet pulp, maize gluten feed, brewers grains, distillers dark grains), concentrate feed (CON), oil supplements (OIL) and minerals and vitamins supplements (MIN). The proportion of non-Holstein/Friesian cows (BRE) in the herd was used as an indication of breed difference. RDA analyses were performed using the CANOCO package (Ter Braak & Smilauer 1998), with automatic forward selection of variables significance calculated by Monte Carlo permutation tests.

RESULTS

All differences discussed are significant, associated with ANOVA P-values < 0.05, unless stated otherwise.

Milk Yield and Basic Composition

As expected, the performance in milk yield and fat and protein content (Table 1) differed between the three systems of contrasting production intensity and between the two years. Milk production expressed as kg/cow/day and energy corrected milk (ECM; (Peterson *et al*. 2012)) in CH farms was higher than OM and CL farms. On the other hand, milk fat and protein content were lowest in CH milk, highest in CL and showed intermediate values in milk from OM farms. Milk urea was higher during year 1 of the study milk but milk protein and SCC were higher during year 2. Mastitis treatments were higher during year 1. Details of seasonal variation in milk yield, basic composition and mastitis and other veterinary treatments are presented in appendix (Table S1).

When the relationships between swards productivity/botanical composition and milk yield, basic composition and mastitis and other veterinary treatments were investigated by RDA (Figure 1a), positive associations were detected between (i) herbage yield and milk yield along axis 1, (ii) proportion of plants other than grass and clover in the swards with milk SCC and fat content along axis 1 and (iii) grass proportion in the swards with milk fat, protein and urea content along axis 2.

When the relationships between cows' diet/breed choice and milk yield and basic composition and mastitis and other veterinary treatments were investigated by RDA (Figure 2a) positive associations were found for (i) milk protein and fat contents and pasture intake and the use of alternative breeds along axis 2, (ii) the use of concentrates and milk urea along axis 2 and (iii) the use of by products and alternative breeds with SCC. The use of alternative breeds and pasture intake were negatively associated with mastitis and other veterinary treatments but also with milk yield which was in turn positively linked with feeding silage, cereals and concentrate feeds.

Fatty Acid Composition

Significant impacts of production intensity and year were identified for milk FA profile (Table 2). Milk from CH farms was higher in palmitic acid, SFA and had a higher atherogenicity index (AI; as described by Ulbricht and Southgate (1991)) and n-6:n-3 ratio whereas it was lower in stearic acid, MUFA and PUFA relative to other systems. Milk OA concentrations were higher in CL than CH milk while OM milk did not differ from either systems. Milk VA and RA content were elevated with increasing pasture intake (CH < OM < CL). The highest concentrations of n-3 were seen in OM milk, the lowest in CH with intermediate values in CL milk. Milk n-6 concentrations were higher in CH and OM milk than in CL. Milk collected in year 1 had more OA, VA, RA and MUFA and less lauric acid, myristic acid, palmitic acid, stearic acid, SFA and lower AI compared with milk from year 2. Details of seasonal variation in milk FA composition are presented in appendix (Table S2).

Interactions between production intensity and sampling month were significant for palmitic acid, VA, RA, and n-3, shown in Figure 3, and for lauric acid, myristic acid, stearic acid, OA, SFA, MUFA and AI, shown in appendix (Figure S1). Milk from CH farms had more palmitic acid throughout the study than CL milk and also compared with OM milk during March and May. Milk from CL and OM farms had more VA and RA than CH milk throughout the study, and in August, the difference between CL and OM farms also reached significance, with milk from the former being higher in VA and RA. Similar findings were seen for n-3 although in this case it was OM milk showing higher concentrations than CL milk in August. Milk from OM and CL farms had lower n-6:n-3 ratio than milk from CH farms with relative differences being greatest in March and October.

Significant interactions between production intensity and year were detected for myristic acid (appendix, Figure S2), RA, n-3 and n-6 concentrations in milk and n-6:n-3 ratio (Figure 4). Milk from CH farms had less RA which was consistent across the two years whereas both OM and CL milk showed a decrease from the first to the second year of the study. Concentrations of n-3 in milk did not differ between years in any of the systems but n-6 in OM milk was higher in the second than in the first year. Milk from CH farms showed lower n-6:n-3 ratio in year 2, compared with year 1 but this was not found in milk from OM and CL farms.

RDA (Figure 1b) showed a positive association between the relative proportion of grass and other plants in the swards with milk stearic acid, OA and MUFA and to a lesser extent with VA and n-3, while individual and total SFA were positively linked with herbage yield and clover content, along axis 1. When dietary components and breed choice were compared with milk FA profiles by RDA (Figure 2b) stearic acid, OA, VA, RA, MUFA, PUFA and n-3 were positively associated with predicted pasture intake and the use of breeds other than Holstein/Friesian and negatively linked with other feeds including silage, hay/straw, cereals, concentrate feed and oil supplementation, along axis 1 in Figure 2b. The latter feeding practices were positively linked to milk palmitic acid, SFA and AI, and to a lesser extent lauric acid, myristic acid, n-6 and n-6:n-3 ratio.

Antioxidant Composition

As with FA profiles, differences were detected for α-tocopherol and all antioxidants assessed in milk, influenced by system of production and year of sampling, as shown in Table 2. Contents of α-tocopherol were higher in CL than CH milk while OM milk did not differ from either of the other systems. Lutein, ß-carotene and total carotenoids concentrations in milk followed pasture intake (CH < OM < CL) while more zeaxanthin was found in CL compared with CH and OM milk. Milk collected in year 1 had more α-tocopherol, ß-carotene, lutein and zeaxanthin than milk from year 2. Details of seasonal variation in milk antioxidant composition are presented in appendix (Table S2).

Interactions between production intensity and sampling month were detected for ß-carotene, lutein and total carotenoids, as shown in Figure 3, and α-tocopherol, as shown in appendix Figure S1. Lutein, ß-carotene, and total carotenoids were consistently higher in CL than in CH milk throughout the study and higher than OM milk in August and October. OM milk contained more (i) lutein in March and October and (ii) ß-carotene and total carotenoids in March, compared with CL milk.

Interactions between production intensity and year were detected for concentrations of α-tocopherol, lutein, zeaxanthin, ß-carotene (Figure 4) and total carotenoids (appendix, Figure S2). Higher contents of α-tocopherol in OM and CL compared with CH milk observed in year 1 were not confirmed during year 2, when OM milk was higher than CL milk (Figure 4). In year 1, lutein and zeaxanthin content rose with increased pasture intake (CH < OM < CL) although these relations were not all significant in year 2, when (i) concentrations of lutein in OM and CL were similar but still higher than CH, (ii) only OM had more zeaxanthin than CH milk (Figure 4) and (iii) only CL milk had more total carotenoids than CH milk (Figure S2). Milk content of β-carotene dropped between first and second year only in CL farms thus eliminating the higher values found in year 1 when compares with CH and OM milk (Figure 4).

RDA (Figure 1c) revealed milk α-tocopherol and carotenoids were positively linked to herbage yield and proportion of clover and other plants in the swards. The use of alternative genetics and predicted pasture intake were positively associated with milk β-carotene, lutein and total carotenoids, along axis 2, while α-tocopherol and zeaxanthin content were negatively associated with the proportion of maize silage, concentrate feeds and oil supplementation in the diet, along axis 1 (Figure 2c).

Protein Composition

Table 3 shows how milk protein composition was influenced by production systems and season. Total protein, casein, and whey proteins, αCN and βCN were higher in CL compared with CH and OM milk. Concentrations of κ-casein increased with higher pasture reliance (CH < OM < CL) and the ratio of casein:whey proteins was higher in CL and OM milk than in CH milk. Whereas the overall effect of production intensity on total βLg content of milk appeared significant in the ANOVA, mean values determined by TSHD test proved not to differ. March milk was higher in protein, αCN, βCN and κCN compared with that produced in May, June and August and total casein was higher in milk collected in March and May compared with August. Concentrations of whey protein, βLg and its variants, A and B, were lower in March and June and increased over the months of August and October. The casein:whey protein ratio was highest in June, intermediate in March and May and lowest in August and October. On the other hand, αLa was highest in March, intermediate in May-August and lowest in October samples.

Interactions between production intensity and sampling month were significant for all individual proteins and protein classes (Figure 5 and appendix Figure S3). Total protein concentrations were higher in milk from CL farms compared with CH milk throughout the survey except for May when the contents were similar (Figure 5). Total protein in OM milk was higher than in CH milk during October and lower than CL milk in August and October with similar patterns seen for milk casein concentrations (Figure 5). The casein:whey protein ratio was higher in milk from CL farms compared with CH farms in March and August (Figure 5).

When the relationships between milk protein composition with sward characteristics were determined by RDA (Figure 1d), the proportion of clover and herbage yield were negatively linked with total protein and individual and total caseins, along axis 1. In addition (Figure 2d), all individual proteins (except for βLgA) and the casein:whey protein ratio were positively associated with alternative breeding and predicted pasture intake and negatively associated with inclusion of silage, cereals, by-products and hay/straw in the diet, along axis 1.

DISCUSSION

Effect on Milk Yield and Basic Composition

CH herds, dominated by Holstein/Friesian cows fed more silage and concentratesthroughout the year, produced more milk than herds milking crossbred cows on a predominantly grazing system, as supported by other authors (White *et al.* 2001; Carroll *et al.* 2006; Stergiadis *et al.* 2012). However, despite higher supplementation, grazing was still important to these farms and the RDA revealed a very close association between herbage yield from grazing paddocks and daily milk yield per cow; pointing out the importance of optimum grass management in pasture-based herds to maximise output. The inclusion of Jersey genetics in OM and CL herds is likely to contribute to lower milk output and explain the higher milk solid content for these herds (White *et al.* 2001; Croissant *et al.* 2007; Stergiadis *et al.* 2013). A decrease in milk fat content from cows under pasture-based diets has been shown by other studies (White *et al.* 2001; Croissant *et al.* 2007) but in this study it seems the impact of genetics is stronger than the possible negative influence of low fibre, leafy herbage on milk fat content.

Since OM and CL herds block calved in spring, all cows were in early lactation in March and late lactation in October, making it impossible to differentiate between seasonal and stage of lactation effects on milk quality, for 10 of the 15 herds in this study. This could partly explain overall patterns in milk yield, peaking in May and being lowest in October. Milk fat and protein content followed a contrasting pattern throughout the year which may be partly explained by the negative correlation between milk yield and solids content (Chalupa & Sniffen 2000).

Interestingly, the proportion of cows showing signs of mastitis was numerically higher in CH than OM and CL herds, despite greater use of preventive antibiotic treatments which are not allowed in organic farming and scarcely used in CL systems, although the difference was not statistically significant. However, mastitis risk is influenced by a wide range of confounding factors and, in this study, it is difficult to explain this effect. The finding that pasture intake and alternative genetics are negatively associated with mastitis and other veterinary treatments, revealed by RDA analyses, is in line with previously published work showing lower clinical and subclinical rates of mastitis in grazing cows (Goldberg *et al.* 1992).

Effect on Fatty Acid Composition

Lower production intensity has been shown to improve milk FA profiles by reducing SFA and increasing MUFA and PUFA (Butler *et al.* 2008; Slots *et al.* 2009; Stergiadis *et al.* 2012) and the same was observed in this study. Nutritionally undesirable SFA, including C16:0, were lower in milk from OM and CL farms; whilst unsaturated FA, beneficial for human health, such as OA, VA, RA and n-3 were considerably higher. Compared to published composition of conventional retail milk in NE England (Butler *et al.* 2011), milk from CL farms in this study had 60% more n-3 and 2.75 times more RA. Similarly, OM milk in this study contained 17% less SFA, 15% more n-3 and 62% more RA than comparable values reported for retail organic milk (Butler *et al.* 2011), indicating wide variation in management (including pasture reliance) and hence milk composition within apparently similar production systems in the same country. These differences reinforce the opinion that aspects of quality relies more on dairy diets rather than the production system *per se* and sustaining high pasture intake improves milk quality both in conventional and organic systems. To some extent, prescribed standards ensure organic producers follow fairly similar practices but variation in conventional management is much greater from pasture-based at one extreme to intensive systems with year round housing at the other. Hence the scope to improvemilk quality by encouraging pasture intake seems greater in conventional milk production.

The desirable impact of fresh grass intake on milk FA composition has been reviewed (Elgersma *et al.* 2006) and confirmed by RDA in this study. Fresh grass is richer in PUFA, especially ALA, compared to conserved forages (Glasser *et al.* 2013) and its intake increases the appearance of ALA, products of PUFA rumen biohydrogenation and RA in milk (Chilliard *et al.* 2000). However, positive associations, shown by RDA, between (i) proportion of grasses in pasture with milk OA and MUFA and (ii) n-3 in milk with the proportion of plants other than grasses and clover in the pastures, confirms botanical diversity has an additional role in improving milk FA profiles.

Milk from both OM and CL systems showed more desirable FA profile than CH milk but there were also differences between these 2 systems. For example, CL milk was higher in VA and RA compared with OM milk, possibly reflecting the higher pasture intake on CL farms (Butler *et al.* 2008) exacerbated by lower rates of hydrogenation (less VA production) associated with clover inclusion in the OM swards (Dewhurst *et al.* 2009; Lee *et al.* 2009; Lejonklev *et al.* 2013). This finding is particularly interesting because Stergiadis et al. (2012) reported that raising pasture intake from 200 g/kg to 370 g/kg of DMI throughout the year did not have a significant impact on VA and RA contents of milk but the similar increase (albeit from a much higher baseline) in pasture intake in this study (700 g/kg DMI for OM vs 840 g/kg DMI on CL farms) did significantly raise milk VA and RA content by 21% and 28% respectively. These differences were more pronounced comparing milk from CH and OM farms; VA and RA content were approximately 2.1 and 1.9 times higher when pasture intake increased by 270 g/kg DMI (430 to 700 g/kg DMI). This suggests the response in milk composition from increased pasture reliance may be more apparent when a high proportion of cows' diet already comes from fresh grass (possibly above the 430 g/kg DMI recorded in this study). Another explanation could be that, feed components, other than fresh grass, have a greater negative influence on RA when pasture intake is moderate or low. Milk from OM farms showed the highest n-3 content despite the medium pasture intake, most likely explained by the inclusion of clover and other non-grass plants in organic swards. Clover can increase n-3 content of milk by both increasing n-3 intake and reducing its ruminal biohydrogenation (Dewhurst *et al.* 2009; Lee *et al.* 2009; Lejonklev *et al.* 2013). However, RDA in this study showed that the presence of 'plants other than grass or clover', a practice more common in organic farming in UK, may have a greater impact on milk beneficial FA than clover itself. This is in line with studies in alpine regions showing increasing botanical diversity of the swards has a positive impact of milk FA profiles, by raising contents of beneficial FA such as MUFA and PUFA (Collomb *et al.* 2008). Thus, when comparing FA profiles of OM and CL milk, both show advantages; milk from OM herds was higher in n-3 (as a result of botanically diverse swards) whereas CL milk was higher in RA (as a result of greater reliance on grazing).

Although all milk types in this study had n-6:n-3 ratio within the recommended 1-4:1 range (Simopoulos 2002), the lower ratio of OM and CL milks can be considered more desirable. Western diets are dominated by n-6, overconsumption of which may increase the risk of CVD, cancer, and inflammatory and autoimmune diseases (Simopoulos 2002). Although a threshold of 2.3, under which no further CVD-prevention benefits may be expected, has been suggested in other studies (Benbrook *et al.* 2013) individual foods with a n-6:n-

3 ratio within the recommended range or lower would help sustain the recommended ratio for total diet and compensate for high n-6 intakes from other sources. Notably, n-6:n-3 ratios of milk from pasture-based systems in this study were over 2.5 times lower than reported values for conventional (1.40 vs 3.70) and over 1.5 times lower for organic (1.65 vs 2.70) UK retail milk (Butler *et al.* 2011).

Effect on Antioxidant Composition

As suggested by RDA, the higher contents of antioxidants in milk from OM and CL systems can largely be explained by a combination of greater estimated intake from pasture and a higher proportion of Jersey genetics in these herds; two parameters that may have a synergistic effect. Consequently, CL milk showed the highest concentrations of lutein, ß-carotene and total carotenoids throughout the year. The antioxidants content of milk reflects its availability in cows' diets (Noziere *et al.* 2006; Agabriel *et al.* 2007) while alternative breeds such as Jersey, Guernsey, Ayrshire, Shorthorn have been shown to more efficiently transfer antioxidants from diet to milk compared with Holstein/Friesian cows (Noziere *et al.* 2006). Since the carotenoid content of forages declines during ensiling (20-80% loss) or haymaking (83% loss) and maize silage and concentrate feeds (used in higher amounts in CH farms) are both relatively low in antioxidants (Noziere *et al.* 2006), it was not surprising that CH milk was the lowest in antioxidants. Results from RDA in this study confirm that milk carotenoid content was (i) mainly influenced by pasture intake and breeds used and (ii) more positively influenced by plants other than grass and clover in the swards, implying an extra advantage on OM farms.

All milk collected in this study was relatively high in antioxidants. To our knowledge, there are no studies reporting details of antioxidant content of milk available to the public in UK. In comparison to a previous UK farm-based survey in (North East England; (Stergiadis *et al.* 2012)) results from pasture-based production in South Wales showed CL milk had 2-5 times more lutein, zeaxanthin and total carotenoids and 42-61% more α-tocopherol than conventional milk in North East England. Milk from OM farms had 80% more lutein, 140% more zeaxanthin and 30% more α-tocopherol than organic milk in North East England (Stergiadis *et al.* 2012). These differences between the studies confirm, as with FA profile, the high variability in milk quality that exists within production systems possibly as a consequence of differences in pasture reliance (due to climatic conditions) and breeds used.

Effect on Protein Composition

The higher casein:whey protein ratio in OM and CL compared with CH milk is a result of higher concentrations of individual caseins (αCN, ßCN, κCN) since individual and total whey proteins were fairly consistent. This is of nutritional importance because caseins and bioactive peptides produced during milk digestion or fermentation have been associated with positive effects on human health (Severin & Wenshui 2005; Korhonen & Pihlanto 2006). This finding may also be relevant for processing, especially cheese making, because milk high in individual caseins and casein:whey protein ratio gives better coagulation, curd formation and eventually cheese yield (Christian *et al.* 1999; Wedholm *et al.* 2006); Guinee *et al.* (1998) reported milk from grazing cows produced 7% more moisture-adjusted Mozzarella cheese when daily herbage intake increased from 16 kg/cow/day to 24 kg/cow/day. However, other studies report a depression in protein content of milk from pasture-based systems in summer, associated with a reduction in grass quality (lower digestibility) (O'Donovan *et al*. 2011); this impact may be less in clover rich swards (OM) due to the lower rate of decline in digestibility as plants mature compared to ryegrass (Dewhurst *et al*. 2009). Low milk protein levels were not observed in systems with a high pasture intake (OM, CL) in this study, again possibly indicating the greater impact of Jersey genetics and the influence of being mid/late lactation with moderate milk yield, since the concentration of milk solids is inversely related to yield. The increase in casein and total protein content of milk from spring-block calving herds at pasture from August to late October has previously been reported by O'Brien (1999) followed by a decline in milk processing characteristics after that period, although samples were not collected that late in lactation in this study.

Herd genetic composition varied between management systems, with the contribution of alternative breeds increasing with decreased production intensity (CH < OM < CL). Alternative breeds, such as Jersey and Ayrshire, more common in the lower intensity systems (OM, CL), are known to have milk with more total protein and casein and a higher casein:protein ratio (Cerbulis & Farrell 1975; Carroll *et al*. 2006; Stergiadis *et al*. 2013). The impact of breed has been described as the most pronounced factor affecting milk protein content (Walker *et al*. 2004; Stergiadis *et al*. 2012; Stergiadis *et al*. 2013), supporting the RDA findings in this study. A negative association between clover content of pasture with individual and total protein and caseins was also found, which could have an adverse effect on milk quality from OM farms but appears to be counterbalanced by the presence of crossbred cows. However, other studies report, clover in pastures has either no impact or increases milk protein content (Murphy & O'Mara 1993; Dewhurst *et al*. 2009) and an apparent negative relationship in RDA may be partially explained by the facts that (i) most clover was found in the OM farms, (ii) CL herds had more Jersey cows than OM and (iii) CH cows received more concentrates (known to increase milk protein content) in their diet than OM cows.

Varying pasture intake may explain differences in protein composition between production systems; an increase in individual casein concentrations in milk (α-casein, β-casein, κ-casein) due to *ad libitum* instead of restricted grazing has been shown by others although changes were inconsistent throughout the grazing season (Christian *et al*. 1999; Mackle *et al*. 1999; Auldist *et al*. 2000).

Milk Quality by Year Interactions

Studies in retail milk show management by year and breed by year interactions affect milk FA and protein profiles and α-tocopherol contents of milk (Butler *et al*. 2011; Stergiadis *et al*. 2013). Although the same was found for most of the beneficial aspects of milk quality relating to OM and CL farms (lower SFA, higher MUFA, PUFA, n-3, OA, VA), milk contents of RA, α-tocopherol, lutein, zeaxanthin and β-carotene were lower for OM or CL herds in year 2, an effect not observed for CH milk, which was consistent across the years.

Despite pasture intake by CH herds being reduced from 550 g/kg DMI in year 1 to only 250 g/kg DMI in year 2 milk RA content did not change significantly. This may reinforce the theory that milk RA concentration is more susceptible to changes in fresh forage intake when a high or very high proportions of the diet come from grazing; Stergiadis et al. (2012) have shown that within a range of relatively moderate intake (200-370 g/kg DMI) there is little impact of pasture intake on RA. However, a closer investigation showed variation between production systems over and above herbage intake; perhaps the existence of this interaction is due to year on year changes in RA content in milk from OM and CL farms, brought about by dietary changes other than pasture intake, such as differing inclusion of hay/straw or other silages although the multivariate analysis does not support this explanation. The lower lutein, zeaxanthin, ß-carotene and total carotenoids content of OM and CL milk in year 2 may be explained by a corresponding decrease in grass silage intake and increase in concentrate supplementation; despite overall similar proportions of pasture intake.

This means, despite OM and CL milk showing a more desirable nutritional profile than CH across both years, optimising individual management (high pasture intake, low intakes of maize silage, cereals hay and concentrates) is important to maximise the positive impact on milk quality and offer consistency across years.

Applications of Pasture-Based Milk in Industry and Human Nutrition

Higher pasture intake results in milk withmore n-3, PUFA, RA, antioxidants, α-tocopherol and caseins which may show benefits on animal health, manufacturing properties of milk and human nutrition. This was consistently over the years and, in most cases, also between sampling month within each year.

The combination of fresh herbage-rich diets along with crossing Holstein/Friesian cows with alternative breeds (Jersey, various "Red" genotypes), has previously been shown to improve welfare, fertility, milk solids and economic efficiency in pasture-based systems (Weigel & Barlass 2003; Sorensen *et al.* 2008). Studies with diets rich in n-3 (through linseed supplementation or inclusion of red clover) have been shown to improve dairy cow fertility (Dewhurst *et al.* 2009) but whether a similar effect may be induced by high n-3 pasture-based diets was not assessed in this study. In terms of milk processing, pasture-based dairying, using alternative breeds can provide milk with desirable casein content (improved cheese making properties) throughout the year, especially if seasonal effects can be eliminated by proper blending of milk from spring and autumn calving herds at critical periods (November-December) (O'Brien 1999). It can also improve butter spreadability by replacing SFA with MUFA and PUFA (Bobe *et al.* 2003). The combined practices of high pasture intakes and crossbreeding improve the antioxidant status of milk; useful both for cows' health and protection against oxidation of the high PUFA levels in pasture-based milk, through the supply chain. Given the multiple benefits, a deeper insight to optimising crossbreeding strategies for certain production systems should be further investigated in large individual animal studies.

This study highlights the scope to market premium quality, pasture-based dairy products, offering an increased supply of nutritionally relevant components; although intakes of beneficial compounds might be enhanced, this study cannot predict an impact on human health. Other studies reported infants consuming organic dairy products experienced reduced eczema incidence compared to those on conventional diets (Kummeling *et al.* 2008). In addition, Benbrook et al. (2013) developed dietary scenarios of replacing dietary non-dairy lipids with full-fat dairy products and emphasized that substituting half of total dietary lipid intake with dairy fat, coupled with avoidance of oils high in LA, would decrease dietary LA:ALA ratio (the two main representative FA in n-6:n-3 ratio) from 10.01 to 5.15 (when using conventional dairy products) or from 7.83 to 4.10 (when using organic dairy products). Ratios of n-6:n-3 were even lower for conventional and organic milk in this study than those used by Benbrook et al. (2013) (1.40 vs 2.52 and 1.65

vs 2.28 respectively), implying that milk produced with a high reliance on pasture could play a significant role in decreasing dietary n-6:n-3 to fall within the recommended level (1-4) and closer to the target ratio (2.3) (Simopoulos 2002; Benbrook *et al.* 2013).

The comparison of SFA contents found in this study with other UK farm-based and retail surveys (Ellis *et al.* 2006; Butler *et al.* 2011; Stergiadis *et al.* 2012) reinforces the opinion that differences within production system may be higher than between production system, depending on individual management practices (primarily diet and breed choice). Average milk contents of SFA extends between 673 and 715 g/kg total FA in different UK dairy production systems, ranging from highly intensive all-year round indoor systems, feeding 500 g/kg DMI from concentrates and milking cows 3-times per day, to pasture based-systems in this study (Ellis *et al.* 2006; Butler *et al.* 2011; Stergiadis *et al.* 2012). This difference is relatively small compared to the threshold of decreasing milk SFA content by 150g/kg total FA before we could expect an impact on public health and reduction on health expenses (Kliem *et al.* 2013).

The lower content of SFA and higher MUFA and PUFA levels were consistent in milk from pasture-based dairy systems of lower intensity. However, milk from non-certified pasture-based farms is not traceable as liquid milk or processed dairy products in the UK market. Due to supply lines, such milk is often bulked with milk from farms under more intensive production (usually supplied from more farms with greater yields) thus diluting the higher content of beneficial n-3, RA, carotenoid and proteins in the supply chain. Although pasture-based production is practiced, consumers can rarely access its milk or other dairy products, making the development of separate production lines, or direct selling of pasture-based milk a consideration for the future. However, pasture-based production is limited by (i) climate and soil conditions and (ii) seasonality (the majority of farms spring-block calve in early spring and do not produce milk in winter). Methods to improve (i) winter milk FA profiles by oilseed supplementation (Glasser *et al.* 2013)add in Stergiadis 2014? and use of clover silages (Dewhurst *et al.* 2009) and (ii) the antioxidant content by feeding different types of silage/conserved forages (Hojer *et al.* 2012; Larsen *et al.* 2013)) are still essential in UK to sustain enhanced quality milk throughout the year. Based on the RDA in this study, maize silage, hay/straw, cereals and concentrate feeds all have a negative impact on milk beneficial FA or carotenoid content or both and their use in winter diets is likely to have an adverse effect to the aim to enhance the nutrition properties of milk.

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Tables

Table 1. Management and production parameters (means ± SE) and veterinary treatments for pasture-based dairy systems of contrasting production intensity (conventional high-intensity [CH], organic medium-intensity [OM], conventional low-intensity [CL]) in two different years (1: 2004/2005, 2: 2007) in Wales

	Production Intensity (I)			Year (Y)		ANOVA P-values*		
Parameters assessed	CH (n=34)	OM (n=40)	CL (n=31)	1 (n=60)	2 (n=45)	I	Y	IxY
Herd size	260 ± 16.1	254 ± 15.4	296 ± 22.5	282 ± 15.2	251 ± 12.8	NS	NS	**<0.001**
% heifers	25 ± 1.3	29 ± 1.3	32 ± 2.2	28 ± 1.1	29 ± 1.6	NS	NS	**0.020**
% new calves	26 ± 3.5	47 ± 7.6	41 ± 8.5	38 ± 5.3	40 ± 6.4	**0.002**	NS	**0.033**
Milk production								
Yield (kg/cow/day)	24.9 ± 0.57	17.6 ± 0.69	17.7 ± 0.69	19.9 ± 0.66	19.8 ± 0.75	**<0.001**	NS	NS
ECM†	27.6 ± 0.62	20.1 ± 0.69	20.5 ± 0.83	22.7 ± 0.70	22.7 ± 0.82	**0.001**	NS	NS
Fat (g/kg milk)	41.0 ± 0.29	43.4 ± 0.71	46.7 ± 0.89	43.2 ± 0.54	44.0 ± 0.75	**0.001**	NS	***0.084***
Protein (g/kg milk)	33.5 ± 0.17	35.0 ± 0.34	36.6 ± 0.43	34.6 ± 0.25	35.4 ± 0.39	**<0.001**	**0.001**	NS
Urea (g/kg milk)	0.39 ± 0.088	0.27 ± 0.016	0.36 ± 0.018	0.37 ± 0.043	0.28 ± 0.012	NS	**0.040**	NS
SCC (x10^3/ml milk)	182 ± 8.1	243 ± 19.4	178 ± 12.7	190 ± 8.6	223 ± 17.6	NS	**0.047**	NS
Nutrition (g/kg DMI)								
Estimated DMI (kg)	18.7 ± 0.25	16.5 ± 0.33	16.9 ± 0.26	17.9 ± 0.20	16.5 ± 0.32	**<0.001**	**<0.001**	**<0.001**
Estimated grazing	426 ± 69.8	695 ± 52.3	843 ± 41.1	695 ± 48.5	594 ± 53.4	**<0.001**	**<0.001**	**0.009**
Conserved forage	326 ± 46.4	131 ± 34.5	58 ± 23.9	156 ± 29.4	195 ± 38.8	**<0.001**	NS	**<0.001**
Grass silage‡	224 ± 33.3	106 ± 27.2	51 ± 22.6	117 ± 22.6	142 ± 28.1	**<0.001**	NS	**<0.001**
Maize silage	53 ± 16.2	10 ± 6.8	2 ± 1.7	18 ± 7.5	26 ± 10.5	**0.003**	NS	**0.009**
Other silage	33 ± 12.5	14 ± 6.7	4 ± 2.9	18 ± 6.1	16 ± 8.2	***0.094***	NS	**0.035**
Hay/Straw	16 ± 5.9	2 ± 1.9	2 ± 1.7	3 ± 1.5	12 ± 4.5	***0.053***	**0.009**	**<0.001**
Cereals	67 ± 12.5	42 ± 10	11 ± 5.2	37 ± 7.5	46 ± 10.2	**0.005**	NS	NS
By products§	79 ± 14.6	55 ± 21.5	21 ± 9.7	49 ± 9.3	57 ± 20.1	**0.010**	NS	NS
Concentrates	102 ± 17.9	76 ± 15.3	67 ± 17.7	63 ± 11.7	107 ± 15.9	NS	**0.003**	***0.091***
Minerals (g/cow/day)	78 ± 13.0	22 ± 9.6	20 ± 7.9	43 ± 8.9	35 ± 9.7	**0.009**	NS	**<0.001**
Veterinary treatments								
Mastitis (% herd)	4.68 ± 0.547	2.37 ± 0.467	2.45 ± 0.900	3.91 ± 0.595	2.11 ± 0.328	NS	**0.014**	NS
Other (% herd)	3.45 ± 0.628	3.78 ± 0.995	1.09 ± 0.398	3.04 ± 0.582	2.64 ± 0.736	NS	NS	NS

*Significances were declared at P < 0.05 (significant, bold) and 0.05 < P < 0.10 (trend, bold-italics); NS: P > 0.10.

† Energy corrected milk = [0.327 x yield (kg/d)]+[12.86 x fat (kg/d)]+[7.65 x protein (kg/d)], as proposed by Peterson et al. (2012)

‡ Conventional silage was made of grass while organic silage was a mixture of organically grown grass and clover

§ products from food and drink manufacture typically; sugar beet pulp, maize gluten feed, brewers grains, distillers dark grains

Table 2. Main effect means ± SE and ANOVA P-values for the effects of production intensity (conventional high-intensity [CH], organic medium-intensity [OM], conventional low-intensity [CL]) and year (1: 2004/2005, 2: 2007) on the fatty acid composition (g/kg total fatty acids) and antioxidants content (mg/kg fat) of milk from pasture-based dairy farms in Wales

Parameters	Production Intensity (I)			Year (Y)		ANOVA P-values[ǂ]		
assessed	CH (n=40)	OM (n=40)	CL (n=36)	1 (n=60)	2 (n=54)	I	Y	IxY
Fatty acids(FA)[†]								
C12:0	36.0 ± 0.86	35.3 ± 1.18	34.4 ± 1.16	32.9 ± 0.76	37.9 ± 0.86	NS	**<0.001**	***0.053***
C14:0	117 ± 1.6	113 ± 2.5	112 ± 2.2	110 ± 1.5	119 ± 1.8	NS	**<0.001**	**0.046**
C16:0	351 ± 6.2	306 ± 5.1	297 ± 4.8	308 ± 5.4	332 ± 5.1	**0.001**	**<0.001**	NS
C18:0	112 ± 2.2	121 ± 3.8	127 ± 2.8	116 ± 2.2	124 ± 2.8	**0.007**	**0.006**	NS
OA	214 ± 3.6	223 ± 5.4	231 ± 4.4	229 ± 3.4	215 ± 4.0	**0.044**	**0.001**	NS
VA	14.2 ± 1.02	30.0 ± 1.47	36.4 ± 1.75	31.5 ± 1.63	20.8 ± 1.43	**<0.001**	**<0.001**	***0.074***
RA	6.3 ± 0.41	12.0 ± 0.62	15.4 ± 0.96	12.7 ± 0.77	9.4 ± 0.63	**<0.001**	**<0.001**	**0.048**
SFA	715 ± 4.6	683 ± 6.0	674 ± 5.5	682 ± 4.8	701 ± 4.8	**0.001**	**<0.001**	NS
MUFA	256 ± 4.2	279 ± 5.8	292 ± 5.0	284 ± 4.4	266 ± 4.3	**0.001**	**<0.001**	***0.098***
PUFA	28.9 ± 0.91	38.1 ± 1.07	35.5 ± 1.01	35.0 ± 0.92	32.9 ± 0.99	**0.001**	***0.051***	***0.067***
n-3	5.9 ± 0.24	10.1 ± 0.29	8.8 ± 0.28	8.3 ± 0.33	8.2 ± 0.31	**<0.001**	NS	**0.042**
n-6	16.7 ± 0.83	16.0 ± 0.98	11.3 ± 0.46	14.1 ± 0.73	15.4 ± 0.70	**0.008**	NS	**0.001**
n-6:n-3	3.07 ± 0.221	1.65 ± 0.115	1.40 ± 0.131	2.04 ± 0.188	2.09 ± 0.136	**<0.001**	NS	**0.001**
AI[‡]	3.05 ± 0.084	2.57 ± 0.090	2.42 ± 0.081	2.51 ± 0.071	2.89 ± 0.078	**<0.001**	**<0.001**	NS
Antioxidants								
α-tocopherol	22.0 ± 0.71	25.2 ± 1.15	26.2 ± 1.82	28.7 ± 0.74	18.6 ± 0.87	**0.013**	**<0.001**	**<0.001**
β-carotene	5.63 ± 0.253	6.58 ± 0.283	8.33 ± 0.443	7.32 ± 0.308	6.06 ± 0.248	**<0.001**	**<0.001**	**0.008**
Lutein	0.45 ± 0.025	0.79 ± 0.038	1.06 ± 0.052	0.80 ± 0.046	0.70 ± 0.045	**<0.001**	**0.023**	**0.020**
Zeaxanthin	0.09 ± 0.008	0.12 ± 0.009	0.16 ± 0.014	0.16 ± 0.007	0.07 ± 0.005	**<0.001**	**<0.001**	**0.002**
Total carotenoids	6.7 ± 0.29	8.2 ± 0.34	10.1 ± 0.47	8.3 ± 0.35	8.3 ± 0.35	**<0.001**	NS	**0.022**

[ǂ]Significances were declared at P < 0.05 (significant, bold) and 0.05 < P < 0.10 (trend, bold italics); NS: P > 0.10.

[†] OA, oleic acid (c9 C18:1); VA, vaccenic acid (t11 C18:1); RA, rumenic acid (c9t11 C18:2); SFA, saturated FA (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0, C24:0); MUFA, monounsaturated FA (c9 C14:1, c9 C16:1, OA, VA, c8 C20:1); PUFA, polyunsaturated FA (c9c12 C18:2, c9c12c15 C18:3, RA, t10c12 C18:2, c8,c11,c14 C20:3, c5c8c11c14 C20:4, c5c8c11c14c17 C20:5, c7c10c13c16c19 C22:5); n-3, omega-3 FA (c9c12c15 C18:3, c5c8c11c14c17 C20:5, c7c10c13c16c19 C22:5); n-6, omega-6 FA (c9c12 C18:2, c8,c11,c14 C20:3, c5c8c11c14 C20:4, c13c16 C22:2, t10c12 C18:2)

[‡] Atherogenicity Index (AI): (C12:0 + 4xC14:0 +C16:0)/(sum of unsaturated FA), as proposed by Ulbricht and Southgate (1991)

Table 3. Main effect means ± SE and ANOVA P-values for the effect of production intensity (conventional high-intensity [CH], organic medium-intensity [OM], conventional low-intensity [CL]) and sampling date (March, May, June, August, October) on the protein composition (g/kg of milk) of milk from pasture-based dairy farms in Wales over year 2 of the study (2007)

	Production Intensity (I)			Sampling Date (D)					ANOVA P-values*		
Parameters assessed	CH (n=25)	OM (n=23)	CL (n=18)	March (n=12)	May (n=13)	June (n=15)	August (n=13)	October (n=13)	I	D	IxD
Total protein	35.9 ± 0.53	37.8 ± 0.50	41.1 ± 0.91	40.1 ± 0.81	37.6 ± 0.54	37.0 ± 0.79	37.0 ± 1.13	38.5 ± 1.36	**0.003**	**<0.001**	**0.025**
Total casein	30.4 ± 0.50	32.4 ± 0.44	35.3 ± 0.80	34.3 ± 0.79	32.4 ± 0.46	32.3 ± 0.72	31.0 ± 1.04	32.4 ± 1.20	**0.003**	**0.001**	**0.039**
Total whey protein	5.46 ± 0.100	5.45 ± 0.151	5.86 ± 0.180	5.83 ± 0.089	5.27 ± 0.103	4.77 ± 0.125	5.96 ± 0.105	6.13 ± 0.167	**0.021**	**<0.001**	**0.003**
Casein:whey	5.61 ± 0.122	6.05 ± 0.184	6.08 ± 0.168	5.89 ± 0.142	6.15 ± 0.077	6.81 ± 0.180	5.19 ± 0.111	5.27 ± 0.072	**0.011**	**<0.001**	**0.050**
α-casein	12.0 ± 0.17	12.9 ± 0.19	14.0 ± 0.34	13.5 ± 0.37	12.7 ± 0.19	12.7 ± 0.29	12.5 ± 0.41	13.0 ± 0.51	**0.002**	**0.013**	**0.024**
β-casein	12.3 ± 0.22	12.8 ± 0.17	13.7 ± 0.29	13.8 ± 0.24	12.8 ± 0.16	12.9 ± 0.26	12.0 ± 0.36	12.8 ± 0.37	**0.016**	**<0.001**	**0.038**
κ-casein	6.08 ± 0.133	6.75 ± 0.113	7.54 ± 0.188	6.97 ± 0.232	6.91 ± 0.143	6.65 ± 0.185	6.50 ± 0.296	6.54 ± 0.328	**0.002**	**0.019**	***0.082***
α-lactalbumin	1.12 ± 0.018	1.14 ± 0.022	1.19 ± 0.040	1.27 ± 0.020	1.18 ± 0.035	1.16 ± 0.029	1.11 ± 0.011	1.02 ± 0.026	***0.051***	**<0.001**	**0.010**
Serum albumin	0.23 ± 0.016	0.22 ± 0.015	0.23 ± 0.015	0.29 ± 0.013	0.30 ± 0.009	0.21 ± 0.010	0.15 ± 0.008	0.19 ± 0.020	NS	**<0.001**	**0.029**
β-lactoglobulin A	1.96 ± 0.087	1.82 ± 0.085	2.16 ± 0.112	1.93 ± 0.081	1.71 ± 0.082	1.59 ± 0.084	2.27 ± 0.099	2.37 ± 0.104	NS	**<0.001**	**<0.001**
β-lactoglobulin B	2.15 ± 0.069	2.27 ± 0.088	2.28 ± 0.096	2.34 ± 0.091	2.09 ± 0.081	1.81 ± 0.064	2.43 ± 0.060	2.54 ± 0.106	NS	**<0.001**	**<0.001**
Total β-lactoglobulin	4.11 ± 0.094	4.08 ± 0.158	4.44 ± 0.198	4.27 ± 0.073	3.80 ± 0.090	3.41 ± 0.099	4.70 ± 0.098	4.92 ± 0.166	**0.026**	**<0.001**	**<0.001**

*Significances were declared at P < 0.05 (significant, bold) and 0.05 < P < 0.10 (trend, bold italics); NS: P > 0.10.

Figure Keys

Figure 1. Biplot derived from the redundancy analysis showing the relationship between herbage yield and botanical composition, as assessed over year 2 of the study (2007), and (a) milk yield (yie), basic composition (fat, prot = protein, urea, scc = somatic cell count), (b) fatty acid profile (c12 = lauric acid, c14 = myristic acid, c16 = palmitic acid, c18 = stearic acid, oa = oleic acid, va = vaccenic acid, ra = rumenic acid, sfa = saturated fatty acids, mufa = monounsaturated fatty acids, pufa = polyunsaturated fatty acids, n-3 = omega-3 fatty acids, n-6 = omega-6 fatty acids, n6n3 = omega-6:omega-3 fatty acid ratio, ai = atherogenicity index as described by Ulbricht and Southgate (1991)), (c) α-tocopherol and antioxidants content (atoc = α-tocopherol, lut = lutein, zea = zeaxanthin, bcar = β-carotene, tcar = total carotenoids) and (d) protein composition (αCN = α-casein, βCN = β-casein, κCN = κ-casein, ala = α-lactalbumin, bsa = bovine serum albumin, blgA = β-lactoglobulin A, blgb = β-lactoglobulin B, blg = β-lactoglobulin). Variables are shown as dots (●) and those being summary of other variables are shown as squares (■). Continuous variables (shown as arrows): DMHA = dry matter herbage per hectar, GRP = ryegrass proportion in herbage, CLP = red/white clover proportion in herbage, OTP = other plants proportion in herbage.

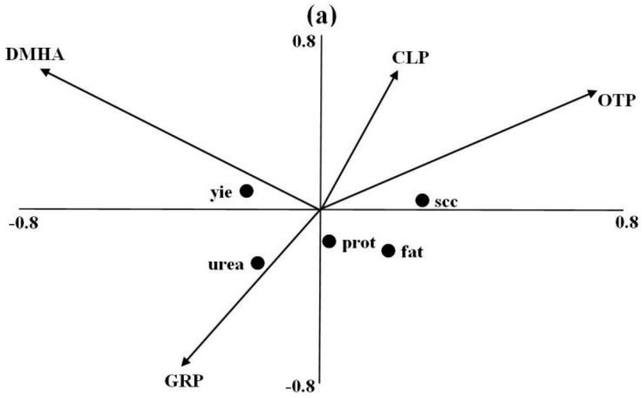
Figure 2. Biplot derived from the redundancy analysis showing the relationship between production system variables, as assessed over the two years of the study (2004/2005 and 2007) , and (a) milk yield (yie) and basic composition (fat, prot = protein, urea, scc = somatic cell count) and veterinary treatments (msp = proportion of mastitis treated cows in the herd, ohp = proportion of other veterinary treated cows in the herd), (b) fatty acid profile (c12 = lauric acid, c14 = myristic acid, c16 = palmitic acid, c18 = stearic acid, oa = oleic acid, va = vaccenic acid, ra = rumenic acid, sfa = saturated fatty acids, mufa = monounsaturated fatty acids, pufa = polyunsaturated fatty acids, n-3 = omega-3 fatty acids, n-6 = omega-6 fatty acids, n6n3 = omega-6:omega-3 fatty acid ratio, ai = atherogenicity index as described by Ulbricht and Southgate (1991)), (c) milk α-tocopherol and antioxidants content (atoc = α-tocopherol, lut = lutein, zea = zeaxanthin, bcar = β-carotene, tcar = total carotenoids) and (d) milk protein composition (αCN = α-casein, βCN = β-casein, κCN = κ-casein, ala = α-lactalbumin, bsa = bovine serum albumin, blgA = β-lactoglobulin A, blgb = β-lactoglobulin B, blg = β-lactoglobulin). Variables are shown as dots (●) and those being summary of other variables are shown as squares (■). Continuous variables (shown as arrows): BRE = % of crossbred and purebred cows of alternative breeds in the herd, GRA = estimated pasture intake, GS = grass silage, MS = maize silage, OS = other silage, HS = hay/straw, CER = cereals, BP = by products (products from food and drink manufacture, typically sugar beet pulp, maize gluten feed, brewers grains, distillers dark grains), CON = concentrate feed, MIN = minerals/vitamins.

Figure 3. Interaction means ± SE for the effects of production intensity (conventional high-intensity [CH], organic medium-intensity [OM], conventional low-intensity [CL]) and sampling month (March, May, August, October) on the (a) concentrations (g/kg total fatty acids) of palmitic acid (C16:0), vaccenic acid (VA), rumenic acid (RA), omega-3 fatty acids (n-3) and n-6:n-3 ratio and (b) contents (mg/kg fat) of lutein, β-carotene, and total carotenoids of milk from pasture-based dairy farms in Wales. P represents the ANOVA P-value for the interaction. Symbols' key: ■ **CH** ▩ **OM** □ **CL**

Figure 4. Interaction means ± SE for the effects of production intensity (conventional high-intensity [CH], organic medium-intensity [OM], conventional low-intensity [CL]) and year (1: 2004/2005, 2: 2007) on the (a) concentrations (g/kg total fatty acids) of rumenic acid (RA), omega-3 fatty acids (n-3), omega-6 fatty acids (n-6) and the n-6:n-3 ratio and (b) contents (mg/kg fat) of lutein, zeaxanthin, β-carotene and α-tocopherol of milk from pasture-based dairy farms in Wales. P represents the ANOVA P-value for the interaction. Symbols' key: ■ **CH** ▩ **OM** □ **CL**

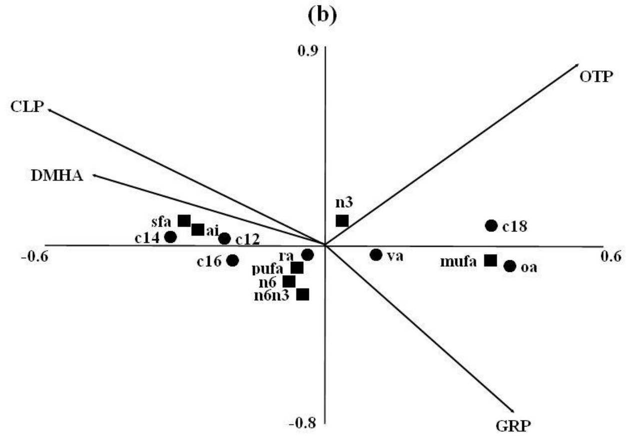
Figure 5. Interaction means ± SE for the effects of production intensity (conventional high-intensity [CH], organic medium-intensity [CH], conventional low-intensity [CL]) and sampling date (March, May, June, August, October) on the contents (g/kg milk) of protein, casein and casein:whey protein ratio of milk from pasture-based dairy farms in Wales over year 2 of the study (2007). P represents the ANOVA P-value for the interaction. Symbols' key: **CH** **OM** **CL**

Driver	F	P
DMHA	1.96	0.174
OTP	1.51	0.136
GRP	0.10	0.742
CLP	Collinear	



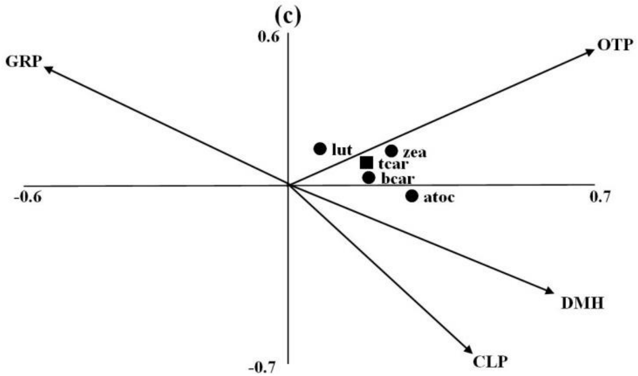
Axis 1 explained 0.07 of the variation and axis 2 explained no further variation

Driver	F	P
GRP	3.21	0.056
CLP	2.15	0.086
DMHA	0.80	0.47
OTP	Collinear	



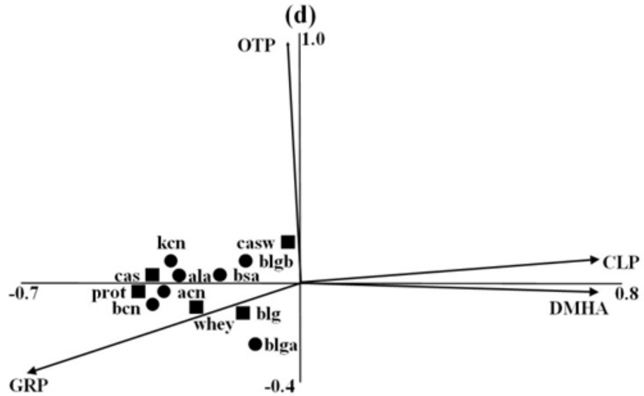
Axis 1 explained 0.08 of the variation and axis 2 a further 0.005

Driver	F	P
OTP	1.84	0.172
DMHA	1.86	0.174
GRP	0.13	0.812
CLP	Collinear	



Axis 1 explained 0.07 of the variation and axis 2 explained no further variation

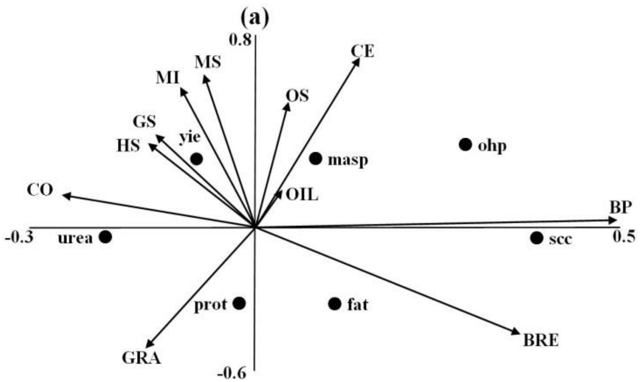
Driver	F	P
CLP	4.18	0.042
DMHA	3.34	0.052
GRP	0.24	0.746
OTP	Collinear	



Axis 1 explained 0.11 of the variation and axis 2 a further 0.003

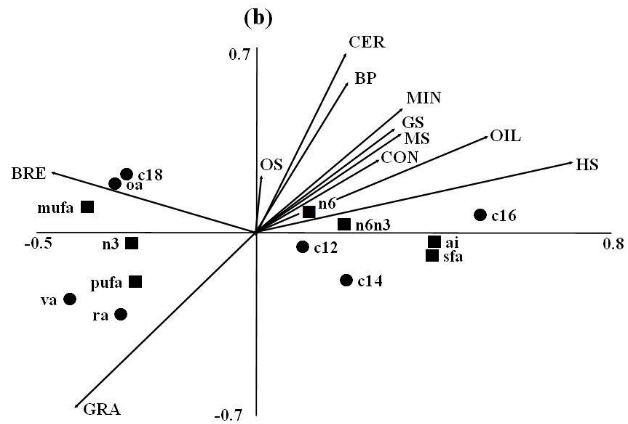
Figure 1

Driver	F	P
BP	3.39	0.032
CON	2.43	0.126
MS	1.99	0.158
BRE	1.93	0.176
OIL	1.13	0.220
GS	0.93	0.280
HS	0.91	0.280
CER	0.58	0.462
GRA	0.39	0.576
MIN	0.38	0.508
OS	0.16	0.654



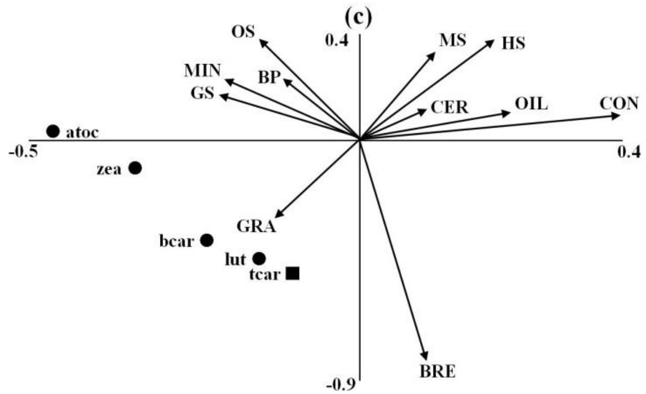
Axis 1 explained 0.15 of the variation and axis 2 a further 0.001

Driver	F	P
HS	10.55	0.002
BRE	4.21	0.022
CER	2.27	0.134
CON	1.86	0.148
GRA	1.76	0.148
OS	1.18	0.296
BP	1.12	0.314
GS	0.65	0.532
OIL	0.42	0.646
MS	0.28	0.724
MIN	0.25	0.750



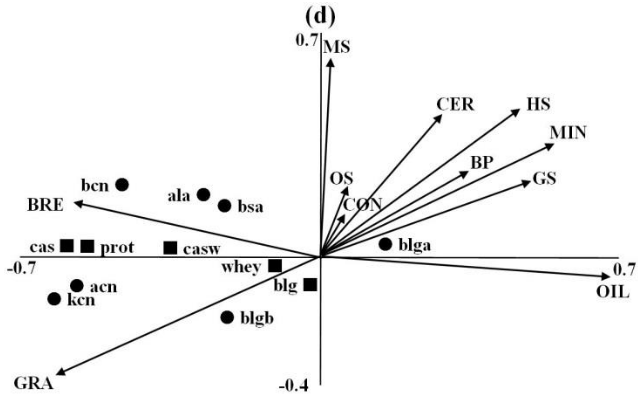
Axis 1 explained 0.18 of the variation and axis 2 a further 0.02

Driver	F	P
HS	4.75	0.028
GS	5.04	0.030
CON	3.43	0.066
CER	3.19	0.076
OIL	2.17	0.118
BRE	1.45	0.182
MIN	1.84	0.188
MS	1.41	0.224
BP	0.70	0.358
GRA	0.56	0.460
OS	0.07	0.856



Axis 1 explained 0.21 of the variation and axis 2 a further 0.01

Driver	F	P
OIL	10.16	0.004
BRE	6.74	0.014
GRA	3.66	0.036
HS	1.82	0.152
COM	0.99	0.272
CER	0.59	0.476
OS	0.45	0.580
BP	0.55	0.500
MIN	0.47	0.588
GS	0.38	0.608
MS	0.37	0.630



Axis 1 explained 0.29 of the variation and axis 2 a further 0.02

Figure 2

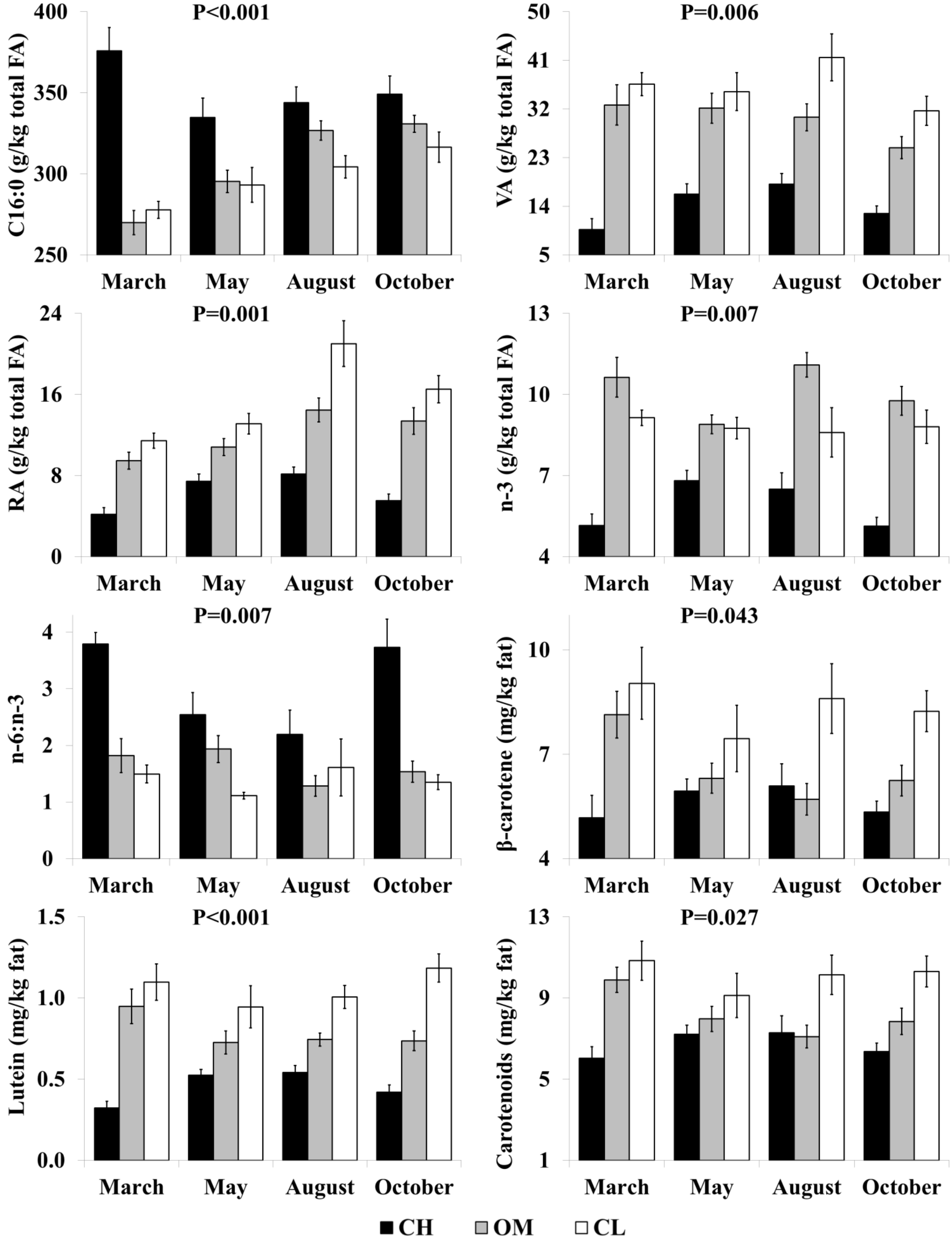

C16:0 (g/kg total FA)
P<0.001
VA (g/kg total FA)
P=0.006
RA (g/kg total FA)
P=0.001
n-3 (g/kg total FA)
P=0.007
n-6:n-3
P=0.007
β-carotene (mg/kg fat)
P=0.043
Lutein (mg/kg fat)
P<0.001
Carotenoids (mg/kg fat)
P=0.027
March
May
August
October
CH
OM
CL

Figure 3

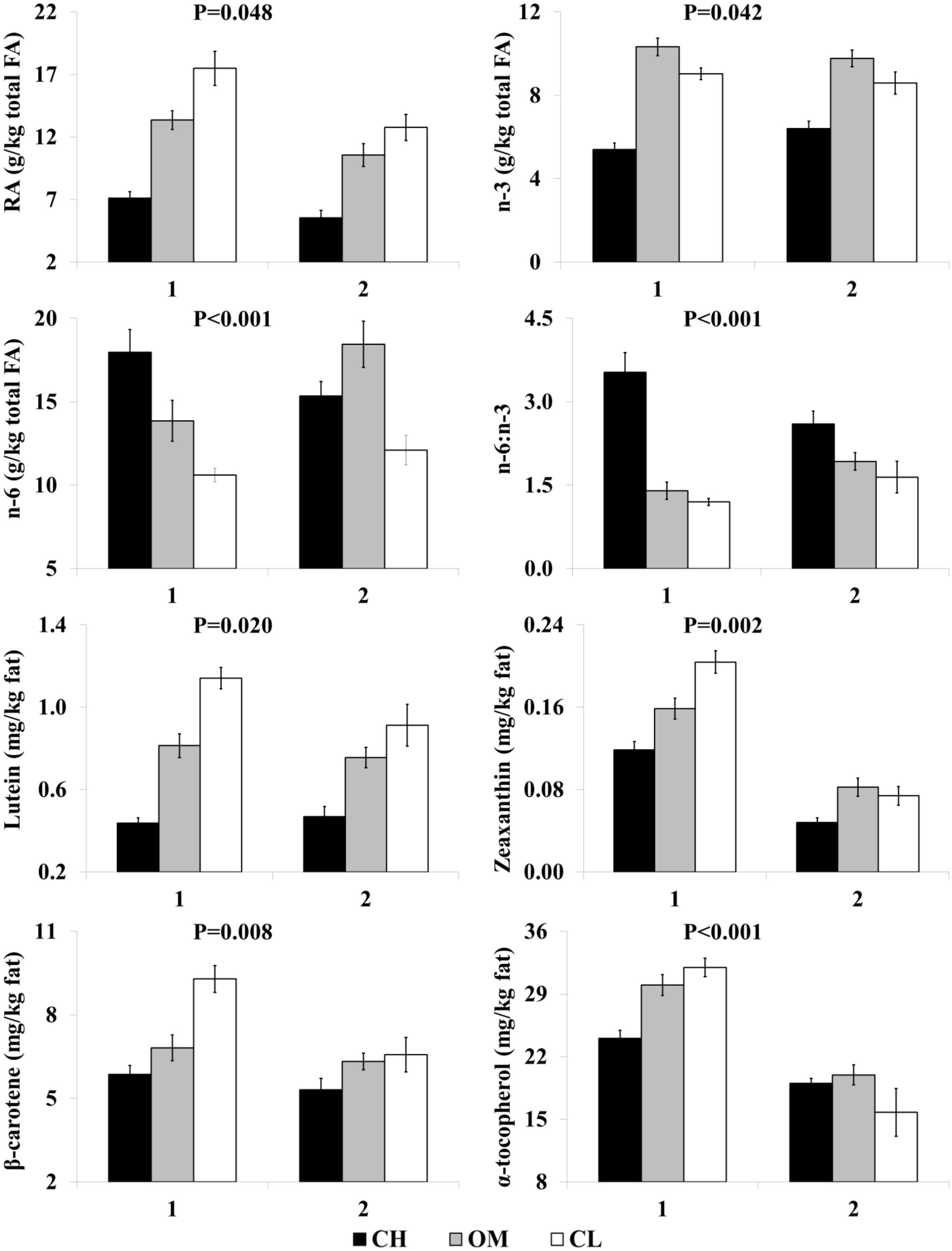

P=0.048
RA (g/kg total FA)
22
17
12
7
2
1
2
P=0.042
n-3 (g/kg total FA)
12
8
4
0
1
2
P<0.001
n-6 (g/kg total FA)
20
15
10
5
1
2
P<0.001
n-6:n-3
4.5
3.0
1.5
0.0
1
2
P=0.020
Lutein (mg/kg fat)
1.4
1.0
0.6
0.2
1
2
P=0.002
Zeaxanthin (mg/kg fat)
0.24
0.16
0.08
0.00
1
2
P=0.008
β-carotene (mg/kg fat)
11
8
5
2
1
2
P<0.001
α-tocopherol (mg/kg fat)
36
29
22
15
8
1
2
CH
OM
CL

Figure 4

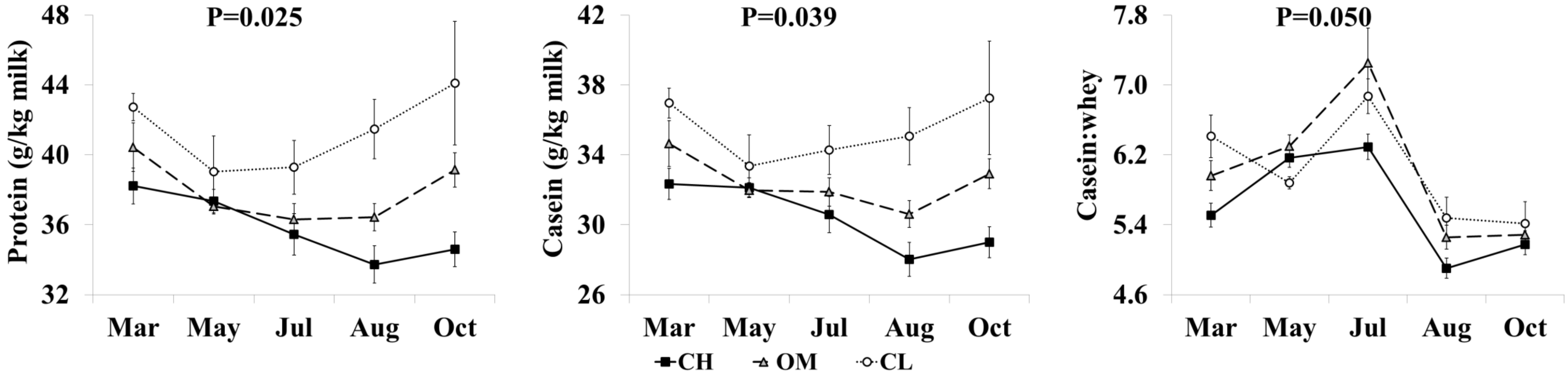

P=0.025
Protein (g/kg milk)
48
44
40
36
32
Mar
May
Jul
Aug
Oct
P=0.039
Casein (g/kg milk)
42
38
34
30
26
Mar
May
Jul
Aug
Oct
P=0.050
Casein:whey
7.8
7.0
6.2
5.4
4.6
Mar
May
Jul
Aug
Oct
CH
OM
CL

Figure 5

Appendix

Table A1. Management and production parameters (means ± SE) and veterinary treatments for pasture-based dairy systems in different sampling dates (March, May, August, October) in Wales

Parameters assessed	March (n=27)	May (n=27)	August (n=25)	October (n=26)	ANOVA P-values[*]
Herd size	247 ± 22.7	283 ± 20.0	271 ± 19.3	273 ± 20.8	NS
% heifers	32 ± 2.3	28 ± 1.6	27 ± 1.4	12 ± 2.1	***0.079***
% new calves	65 ± 7.6	62 ± 8.3	11 ± 4.3	13 ± 4.1	**<0.001**
Milk production					
Yield (kg/cow/day)	21.1 ± 0.85	22.6 ± 0.66	19.1 ± 0.83	16.3 ± 1.20	**<0.001**
ECM[†]	24.2 ± 0.97	24.9 ± 0.78	22.0 ± 0.95	19.5 ± 1.20	**<0.001**
Fat (g/kg milk)	44.0 ± 0.76	39.9 ± 0.58	43.1 ± 0.63	47.2 ± 0.91	**<0.001**
Protein (g/kg milk)	34.2 ± 0.34	33.9 ± 0.26	34.9 ± 0.34	37.0 ± 0.52	**<0.001**
Urea (g/kg milk)	0.26 ± 0.012	0.23 ± 0.017	0.44 ± 0.095	0.39 ± 0.015	**0.003**
SCC ($x10^3$/ml milk)	226 ± 28.9	196 ± 10.2	202 ± 14.2	192 ± 12.7	NS
Nutrition (% DMI)					
Estimated DMI (kg)	17.7 ± 0.46	17.7 ± 0.31	17.2 ± 0.34	16.7 ± 0.37	**0.003**
Estimated grazing	534 ± 81.3	694 ± 73.9	727 ± 59.4	659 ± 68.8	**0.030**
Conserved forage	229 ± 57.7	168 ± 45.7	115 ± 36.3	175 ± 44.8	NS
Grass silage[‡]	147 ± 38.1	117 ± 33.8	97 ± 30.6	149 ± 38.5	NS
Maize silage	42 ± 17.7	32 ± 13.9	7 ± 7.4	2 ± 2.0	**0.034**
Other silage	31 ± 12.0	17 ± 10.6	6 ± 6.5	13 ± 8.9	NS
Hay/Straw	10 ± 5.1	2 ± 1.6	4 ± 2.8	11 ± 6.1	NS
Cereals	55 ± 13.6	42 ± 12.1	28 ± 10.4	37 ± 12.3	NS
By products	94 ± 30.7	34 ± 12.6	39 ± 13.5	41 ± 15.4	NS
Concentrates	87 ± 21.1	62 ± 18.3	91 ± 20.1	88 ± 18.9	NS
Minerals (g/cow/day)	48 ± 15.6	38 ± 11.7	39 ± 13.1	32 ± 12.0	NS
Veterinary treatments					
Mastitis (% herd)	4.38 ± 0.913	3.29 ± 0.798	2.34 ± 0.621	2.46 ± 0.575	NS
Other (% herd)	3.73 ± 1.058	3.43 ± 1.052	2.13 ± 0.751	2.11 ± 0.698	NS

[*] Significances were declared at $P < 0.05$ (significant, bold) and $0.05 < P < 0.10$ (trend, bold-italics); NS: $P > 0.10$.

[†] Energy corrected milk = [0.327 x yield (kg/d)]+[12.86 x fat (kg/d)]+[7.65 x protein (kg/d)], as proposed by Peterson et al. (2012)

[‡] Conventional silage was made of grass while organic silage was a mixture of organically grown grass and clover

[§] products from food and drink manufacture typically; sugar beet pulp, maize gluten feed, brewers grains, distillers dark grains